Yale School of Medicine

Genetic Investigation of Mosaic Skin Disorders Reveals Novel Pathways for Disease Pathogenesis

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Pediatric Dermatology Research Alliance
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Utility of genetics in medicine

To understand of fundamental mechanisms of disease

To enable early diagnosis

To enable disease prevention

To identify new targets for therapeutic intervention

Facts:

> 14 billion human alleles on the planet (Population ~7B)

Most mutations compatible with life are likely present.

Rare recessives often appear due to consanguinity.

Embryonic lethal mutations can appear in mosaic states.

Even very rare diseases in remote locations come to attention.

Despite ~21,000 genes in genome, function known for ~4000

Clinical insight drives discovery – we continue to find "new" disorders and next generation sequencing permits gene discovery.

Approaches to Gene Discovery

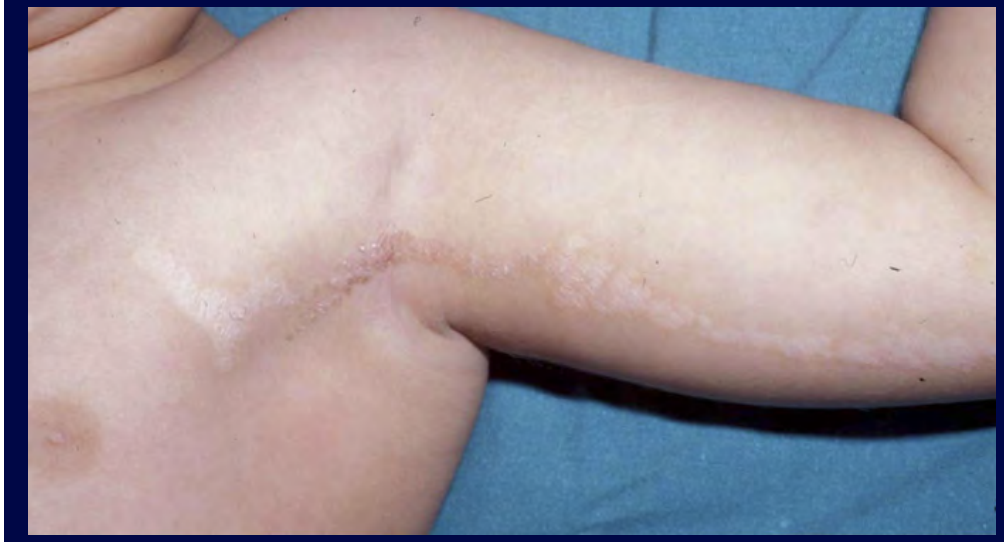
Mosaic patterns occur spontaneously in nature.

All result from mutation during development or subsequent growth.

Timing determines pattern.

Mosaicism in Genetic Skin Disease

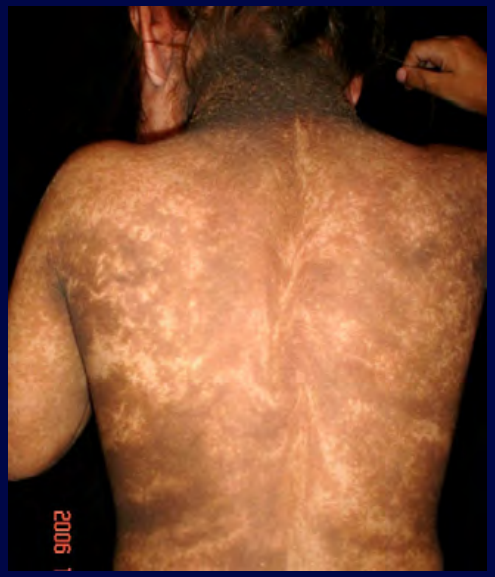
Linear Porokeratosis



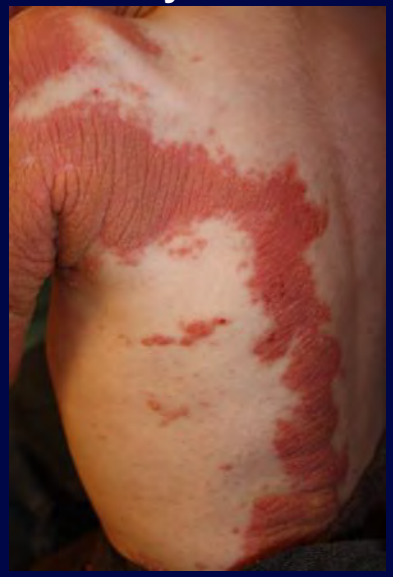
Mosaic conditions typically follow the lines of dorsoventral migration of epidermal precursors.

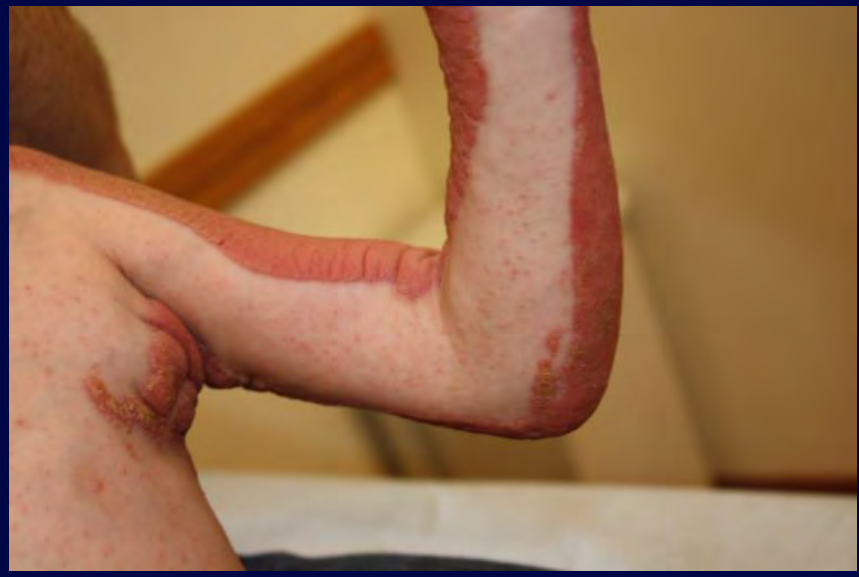
We expect that a single genetic event gives rise to affected skin with timing determining extent and features of disease.

Epidermal Nevus



CHILD Syndrome





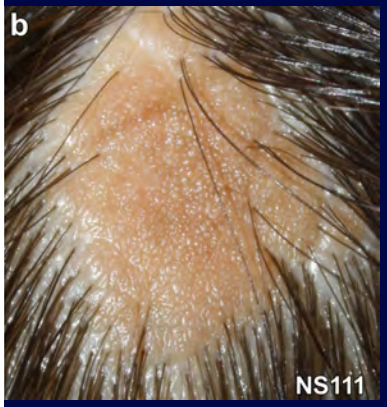
Happle R. *JAAD* 2010.

Approach to Gene Discovery in Mosaic Disorders

1. Determine affected cell type.
2. Isolate affected cell DNA.
   In vitro culture vs. laser capture
3. Isolate unaffected cell DNA and RNA(typically peripheral blood).
4. Perform exome/genome or RNA sequencing using paired DNA or RNA.
5. Identify SNVs present uniquely in affected tissue.

Deleterious mutations (SNVs) unique to affected tissue are likely causative.

Cutaneous Mosaic Disorders Solved



Nevus Sebaceus:
HRAS, NRAS, KRAS

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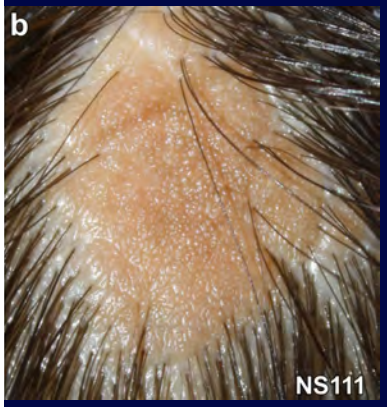

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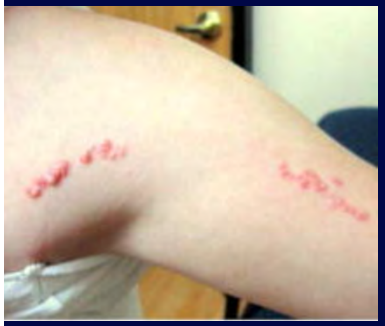

Linear Syringo-cystadenoma Papilliferum:
BRAF

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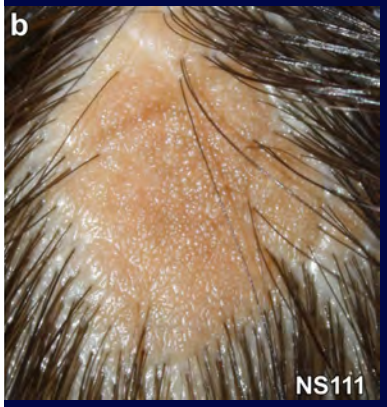

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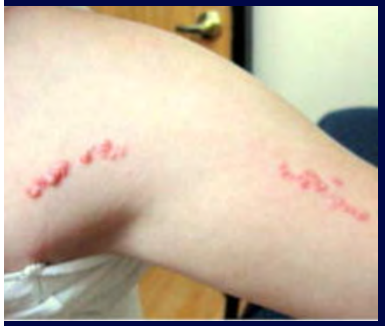

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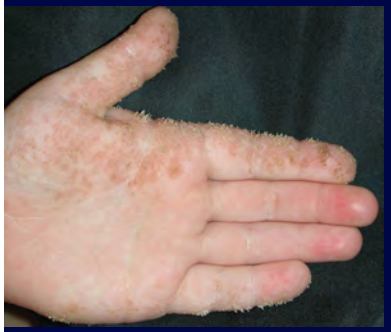

Porokeratotic Ostial and Eccrine Dermal Duct Nevus:
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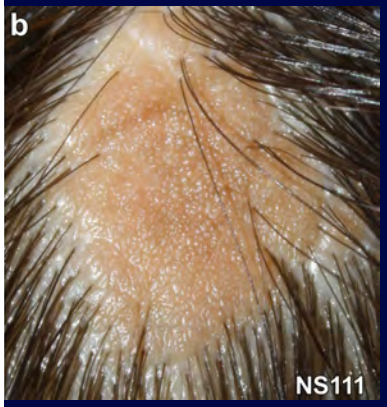

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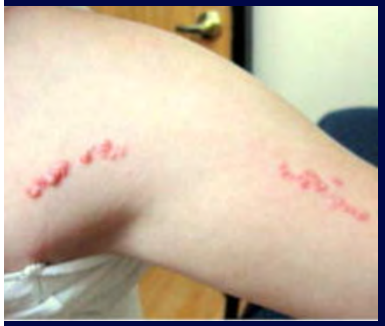

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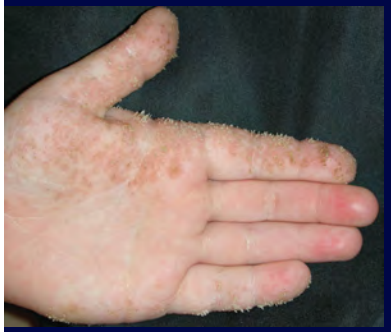

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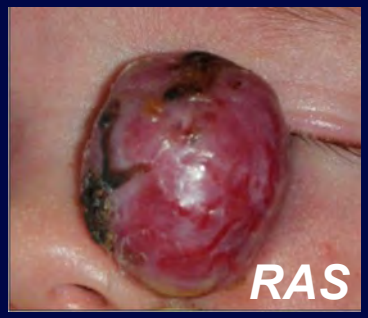

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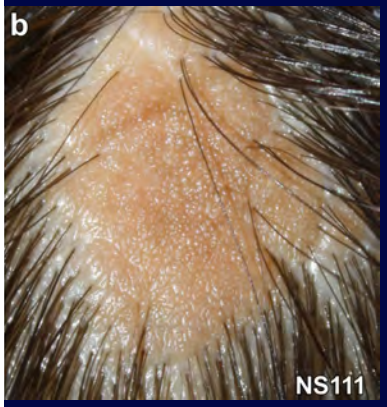

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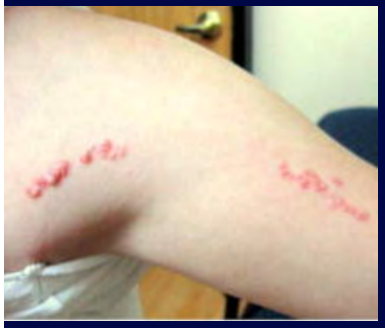

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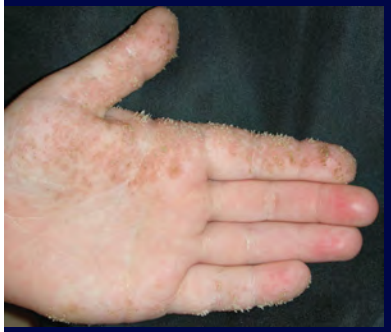

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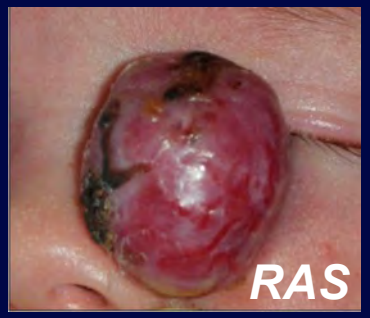

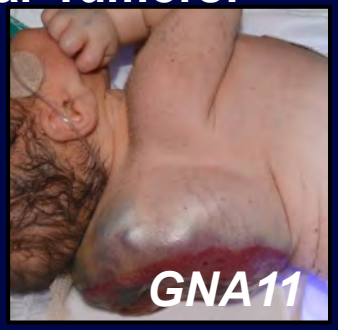

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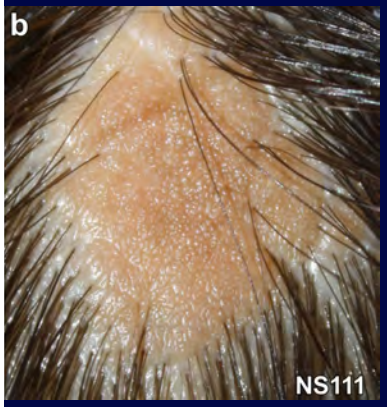

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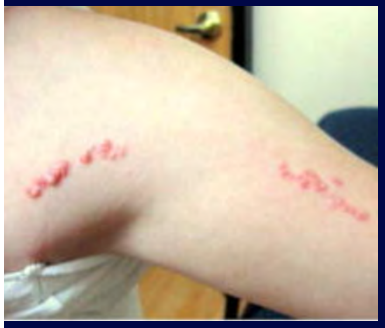

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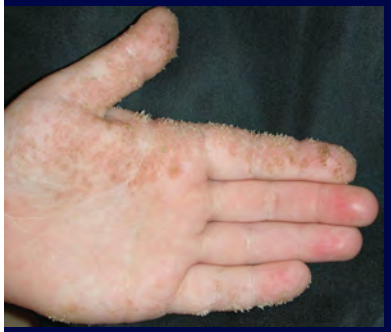

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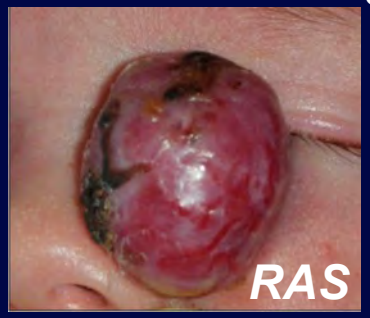

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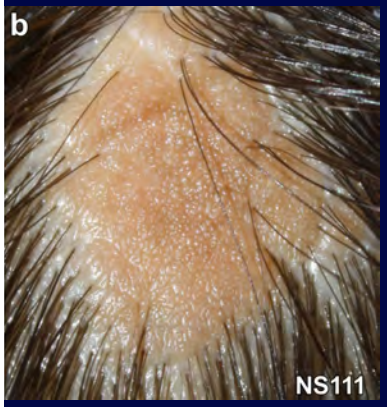

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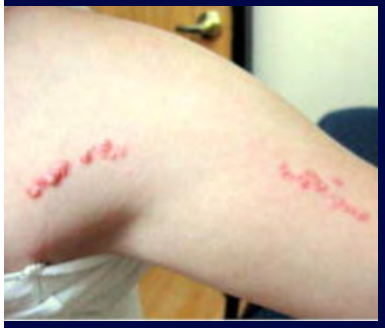

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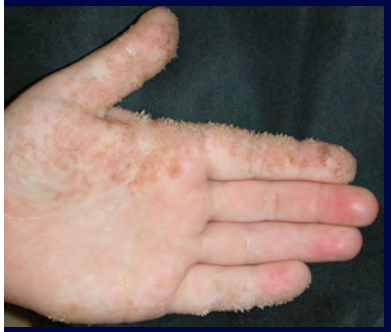

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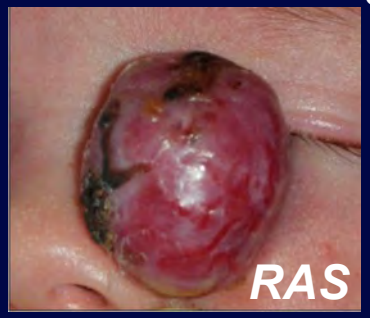

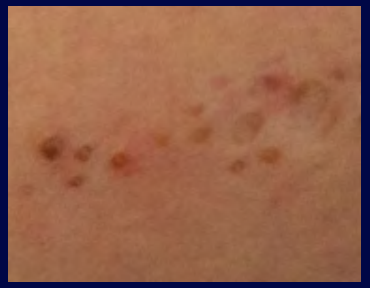

Nevus Comedonicus: *NEK9*

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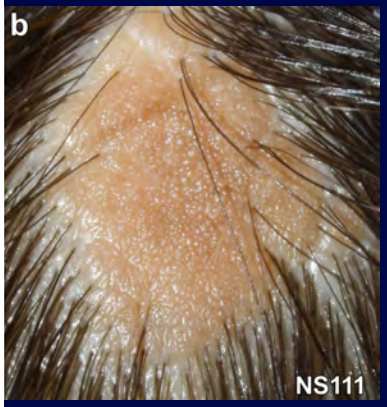

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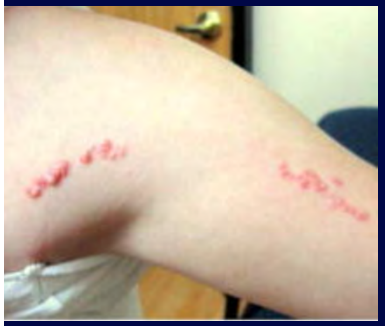

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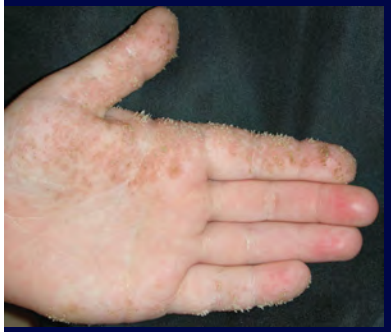

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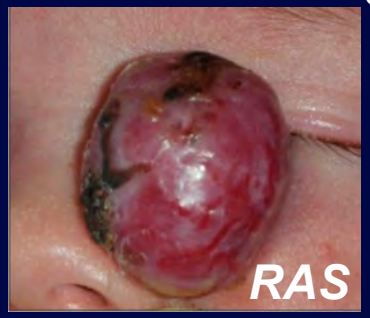

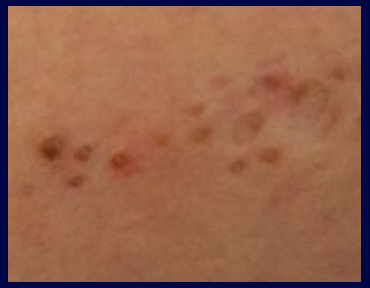

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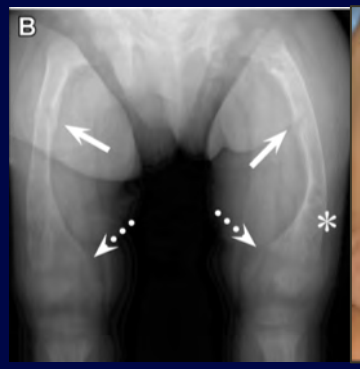
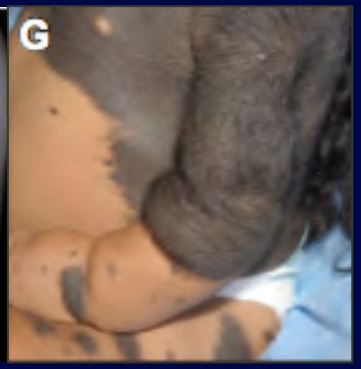




Cutaneous Skeletal Hypo-phosphatemia Syndrome:
H-,K-, N-RAS

New Opportunities for Gene Discovery:

- **Hamartomatous Disorders**
- **Vascular Tumors**
- **Linear/Segmental Inflammatory Disorders**

Hamartomatous Disorders

Many cases of linear disorders remain unsolved:

- Congenital fascial dystrophy
- Nevus Comedonicus (~60% of cases)
- Panfollicular nevi
- Connective tissue nevi

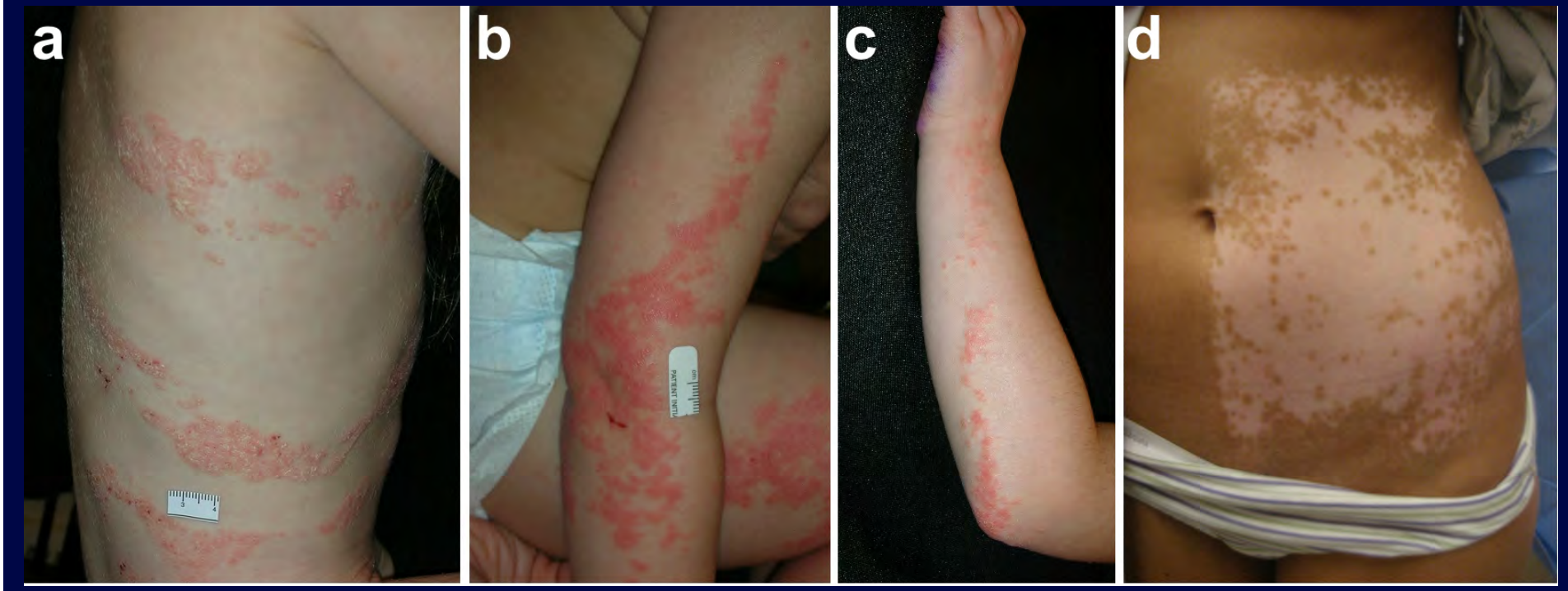
Some types of vascular tumors remain unsolved.

We anticipate that genetic discoveries in these disorders will reveal pathways central to epidermal differentiation, hair follicle specification/renewal, and dermal fibrosis.

Linear/Segmental Inflammatory Disorders

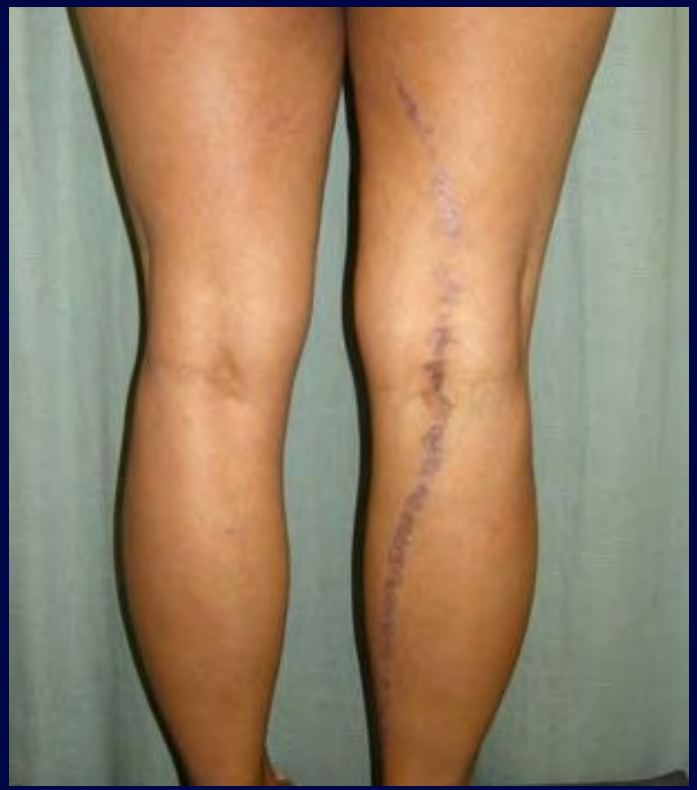
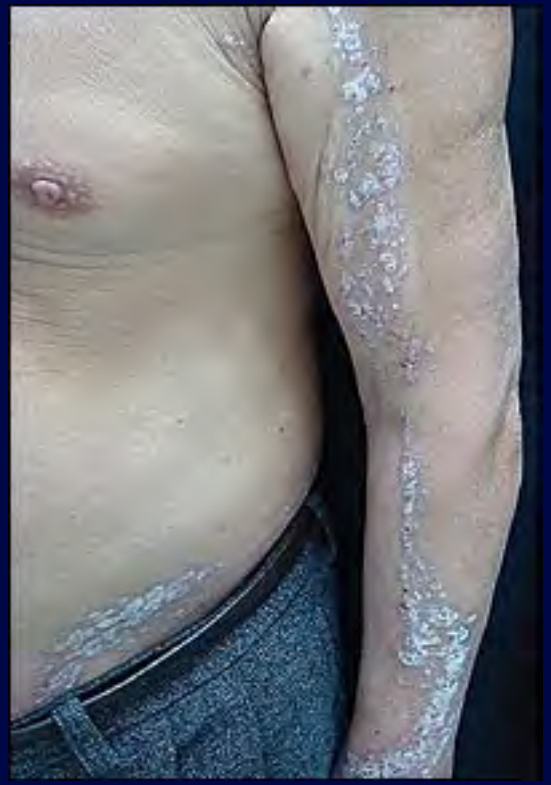
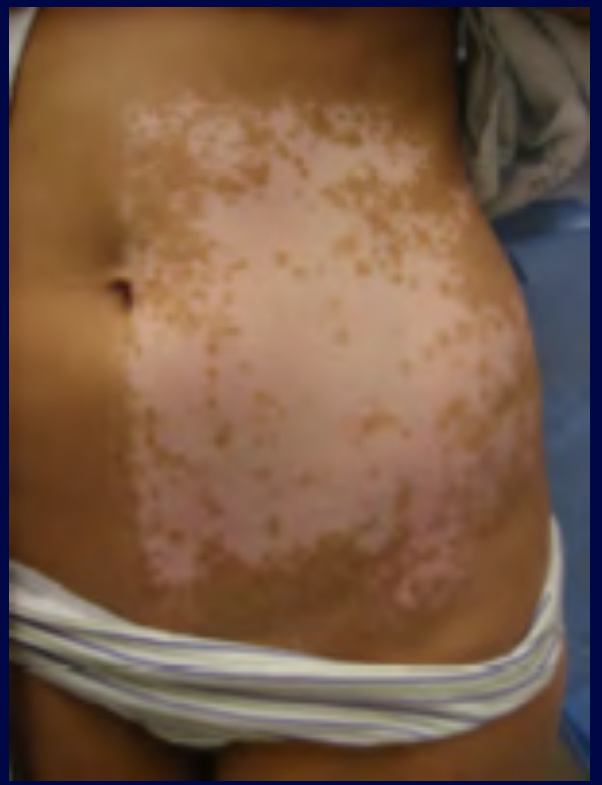
Clinical Characteristics:

- Linear stripes appear early in life
- Usually precede development of generalized disease
- Tend to be more difficult to treat
- Appear in patterns specific to affected cell type



Linear Inflammatory Disorders

- Lichen planus, psoriasis, vitiligo and discoid lupus are canonical inflammatory disorders with mosaic presentations.
- We expect that investigation of these disorders will reveal keratinocytic determinants of inflammation.

Approach

- Collaborating physicians tell subjects about study.
- Our team contacts family, obtains consent.
- Self-service mailer is used to obtain saliva.
- We request block from prior biopsy to core for genetic analysis or a new biopsy is obtained.
- Tissue is pre-screened for mutations in 40 genes using a multiplex amplicon NGS platform:

  AIRE, *AKT1*, *BRAF*, *CARD14*, *CYLD*, *FBN1*, *FGFR1*, *FGFR2*, *FGFR3*, *GJA1*, *GJB2*, *GJB3*, *GJB4*, *GNA11*, *GNA14*, *GNAQ*, *GNAS*, *HRAS*, *KDR*, *KRAS*, *NEK9*, *NRAS*, *IL31RN*, *IL36RN*, *MAP2K1*, *MAP3K3*, *NCSTN*, *NEK9*, *NLRP3*, *NLRP12*, *PIK3CA*, *PSEN1*, *PSENEN*, *PSMB8*, *PTCH1*, *PTEN*, *RASA1*, *RASA2*, *RASA3*, and *TEK*

- Mutation-unknown samples are subjected to exome, genome, and/or RNA sequencing to identify causal mutations

Linear Inflammatory Disorders

Disorders of particular interest:

- **Linear Lupus/Discoid lupus**
- **Linear Lichen Planus**
- **Linear Psoriasis**
- **ILVEN**
- **Segmental vitiligo**
- **Lichen striatus**

Acknowledgements

Mosaic Disorder Gene Discovery

Richard Antaya, Jonathan Levinsohn, Young Lim, Jeff Sugarman, Amy Paller, Moise Levy, Larry Eichenfield, Christine Ko, Jennifer McNiff, Lionel Berkovitch,, Leslie Bostom-Robinson, Ruth Halaban

Please refer cases for genetic diagnosis and gene discovery

Funding:

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National Institute of Arthritis and Musculoskeletal and Skin Diseases

DF

NIH National Human Genome Research Institute

Centers for Mendelian Genomics